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Testing hypotheses of speciation timing in *Dicamptodon copei* and *Dicamptodon aterrimus* (Caudata: Dicamptodontidae)

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Abstract

Giant salamanders of the genus *Dicamptodon* are members of the mesic forest ecosystem that occurs in the Pacific Northwest of North America. We estimate the phylogeny of the genus to test several hypotheses concerning speciation and the origin of current species distributions. Specifically, we test competing a priori hypotheses of dispersal and vicariance to explain the disjunct inland distribution of the Idaho giant salamander (*D. aterrimus*) and to test the hypothesis of Pleistocene speciation of Cope's giant salamander (*D. copei*) using Bayesian hypothesis testing. We determined that available outgroups were too divergent to root the phylogeny effectively, and we calculated Bayesian posterior probabilities for each of the 15 possible root placements for this four-taxon group. This analysis placed the root on the branch leading to *D. aterrimus*, indicating that current distribution and speciation of *D. aterrimus* fit the ancient vicariance hypothesis and are attributable to the orogeny of the Cascade Mountains rather than recent inland dispersal. Furthermore, test results indicate that *D. copei* is distantly related to other coastal lineages and likely originated much earlier than the Pleistocene. These results suggest that speciation within the genus is attributable to ancient geologic events, while more recent Pleistocene glaciation has shaped genetic variation and distributions within the extant species.

Keywords: Bayesian hypothesis testing; Biogeography; *Dicamptodon*; Pacific Northwest

1. Introduction

A current trend in evolutionary biology has been the examination of speciation, current range distributions, and patterns of genetic subdivision in the context of Pleistocene climate change and the associated cycles of glacial advance and retreat (Austin et al., 2002; Church et al., 2003; Crespi et al., 2003; Starkey et al., 2003; Steinfartz et al., 2000; Sullivan et al., 2000; Zamudio and Savage, 2003). Several studies invoke pre-Pleistocene events or conditions to explain patterns of genetic structure, speciation, and disjunct populations in the eastern and southeastern portions of the United States (Austin et al., 2002; Avise and Walker, 1998; Donovan et al., 2000; Zamudio and Savage, 2003) and recently in the western U.S., especially in the Pacific Northwest (Demboski and Cook, 2001; Green et al., 1996; Soltis et al., 1997).

The Pacific Northwest (PNW) of North America has been influenced by numerous geological processes that have resulted in a complex and varied topography. The combination of geologically ancient mountain ranges overlain with recent Pleistocene glaciation provides a complex, yet well-defined historical context with which to interpret genetic data (Cracraft, 1988; Riddle, 1996). As a result, tractable predictive hypotheses are possible with respect to speciation and phylogeography of the

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region (Brunsfeld et al., 2001). Within the PNW, coniferous rainforest ecosystems occur along the western coast of North America, from southern Alaska to northern California, with a disjunct inland forest in the northern Rocky Mountains (NRM) of British Columbia, Idaho, and extreme western Montana. Mesic forests have long been established in the PNW, dating to the mid Eocene in the NRM and were established in their present coastal range by the early Pliocene (5–2 mya; Graham, 1993). The uplift of the Cascades established a rain shadow that caused xerification of the Columbia basin prior to the Pleistocene (2 mya; Daubenmire, 1952; Graham, 1993), which effectively divided mesic forests into a coastal element and an inland element. Subsequent Pleistocene glaciation resulted in severe southern compression of the PNW mesic forests during glacial maxima (Delcourt and Delcourt, 1993; Soltis et al., 1997; Waitt and Thorson, 1983), and would have forced mesic forest organisms into refugia.

The giant salamanders of the genus *Dicamptodon* are endemic to mesic forests of the PNW. Members of this genus provide an ideal study system for examining biogeographic hypotheses since the species are widespread throughout the western United States with several species endemic to particular geographic locales. The genus was originally considered monotypic (Tihen, 1958) but subsequent morphological (Nussbaum, 1970, 1976) and molecular studies (Daugherty et al., 1983; Good, 1989) have resulted in recognition of four species (Fig. 1). *D. copei* is found primarily in the Olympic Peninsula and Coast Range of Washington, *D. ensatus* is restricted to regions surrounding the San Francisco Bay, and *D. tenebrosus* is widespread and ranges from the Cascade Mountains in British Columbia in the north through Washington and Oregon into California. *D. tenebrosus* forms a contact zone with *D. ensatus* north of San Francisco and is sympatric with *D. copei* in parts of western Washington and extreme northern Oregon. The fourth species, *D. aterrimus*, occurs in a disjunct portion of the mesic forest ecosystem in northern Idaho and is geographically isolated from the rest of the genus. Results of allozyme studies have consistently shown that the highest genetic distances within the genus occur between *D. aterrimus* and coastal species (Daugherty et al., 1983; Good, 1989), but relationships within coastal lineages have not yet been resolved (Good, 1989). Here, we use mitochondrial DNA sequence data to resolve relationships within *Dicamptodon* with two complementary analyses. First, we test for monophyly of each of the four described species and second, we test a priori hypotheses regarding speciation for *D. aterrimus* and *D. copei* derived from biogeographic studies in the PNW mesic forest ecosystem.

The competing hypotheses relevant to speciation of *D. aterrimus* in the inland mesic posit either pre-Pleistocene vicariance or post-Pleistocene dispersal (Brunsfeld et al., 2001). The ancient vicariance hypothesis invokes pre-Pleistocene isolation of ancestral *D. aterrimus* from the rest of the genus, associated with xerification of the Columbia basin following the Cascade orogeny. It predicts deep genetic divergence and reciprocal monophyly between *D. aterrimus* and coastal *Dicamptodon* species (Fig. 2A), and requires that *D. aterrimus* persisted in a refuge located in one or more of the river canyons south of glacial maxima throughout the Pleistocene. Phylogeographic studies of two other PNW amphibian lineages endemic to the mesic forests, *Ascaphus trueil/A. montanus* (Neilson et al., 2001) and *Plethodon vandykei/P. idahoensis* (Carstens et al., 2004), provide support for the ancient vicariance hypothesis. Alternatively, *D. aterrimus* could be a post-Pleistocene arrival to the NRM, with either a southern dispersal route through the central Oregon highlands or a northern route through southern British Columbia and northern Washington as glaciers retreated. These inland dispersal hypotheses predict a topology where *D. aterrimus* is nested within the clade from which its ancestors originated: either the clade of northern *D. tenebrosus* haplotypes for the inland dispersal-north hypothesis (Fig. 2B), or southern *D. tenebrosus* haplotypes for the inland dispersal-south hypothesis (Fig. 2C).

A second taxon for which a priori hypotheses have been erected is *D. copei* (Nussbaum, 1976), the only

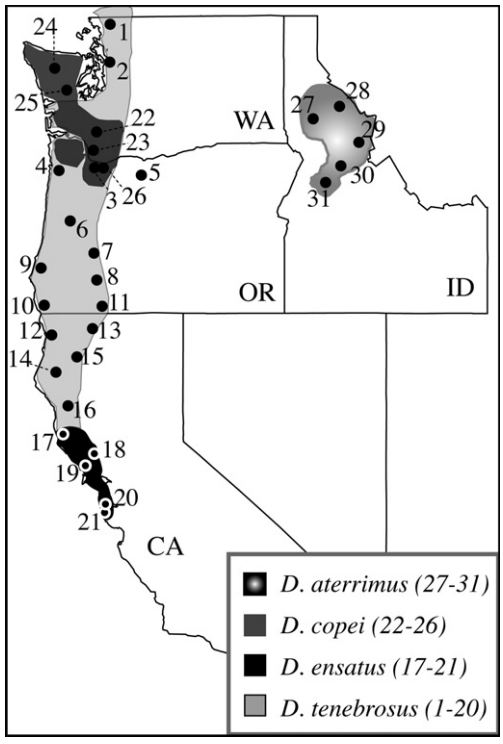

Fig. 1. Approximate distribution of the four species in the salamander genus *Dicamptodon*. Numbers indicate approximate sampling sites and correspond to numbers in Appendix. Populations sometimes included several nearby localities; refer to Appendix for specific locality information.

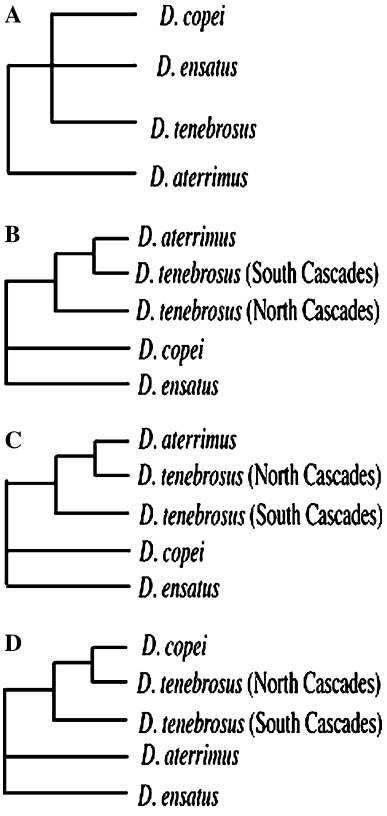

Fig. 2. Constraint trees for phylogenetic hypotheses of interspecific relationships among *Dicamptodon*. The ancient vicariance hypothesis for the speciation of *D. aterrimus* (A) predicts reciprocal monophyly between the inland species of *D. aterrimus* and the reaming coastal clades. (B) The inland dispersal-north hypothesis for the speciation of *D. aterrimus* predicts a close relationship between *D. aterrimus* and northern populations of *D. tenebrosus* (population no. 1–3) while the inland dispersal-south hypothesis (C) predicts a close relationship between *D. aterrimus* and southern populations of *D. tenebrosus*. The Pleistocene-speciation hypothesis for *D. copei* (D) predicts the close relationship between *D. copei* and northern populations of *D. tenebrosus*.

obligate neotene (aquatic gilled adult) within *Dicamptodon*. Nussbaum (1976) proposed that ancestral populations of *D. tenebrosus* occurred throughout western Washington and the Olympic mountains. During Pleistocene glacial maxima, the Puget Sound lobe of the Cordilleran glacier isolated the coastal and Olympic peninsular populations from the northern Cascadian populations of *Dicamptodon*. A harsh terrestrial environment along with abundant pluvial habitat on the coast (Booth, 1987) would have favored an aquatic lifestyle and putatively led to speciation of the neotenic *D. copei* (Nussbaum, 1976). This hypothesis predicts that *D. copei* would be nested within northern populations of a parapyletic *D. tenebrosus* (Fig. 2D). Alternatively, if speciation of *D. copei* predates the Pleistocene, reciprocal monophyly of *D. tenebrosus* and *D. copei* would be predicted, with relatively deep divergence between these taxa.

In this study, we use DNA sequence data to estimate phylogenetic relationships within *Dicamptodon* and explicitly test a priori hypotheses related to the speciation of *D. aterrimus* and *D. copei*. In doing so, we evaluate the relative influence of pre-Pleistocene and Pleistocene geological events on speciation within the genus.

2. Materials and methods

2.1. DNA extraction, amplification, and sequencing

We obtained tissue samples from throughout the geographic range of each species and included: 12 *D. copei* from five populations, 46 *D. tenebrosus* from 16 populations, 10 *D. ensatus* from five populations, and used sequence from six *D. aterrimus* from Carstens et al. (2005) that represented the greatest divergence within this species (Fig. 1; Appendix). Sample sizes ranged from 1 to 5 with an average of 2.8 samples per population. The Ambystomatidae is traditionally considered to be the sister taxon to the Dicamptodontidae (Larson, 1991), and we used sequence from *Ambystoma mexicanum* as a putative outgroup.

DNA was extracted from 10 to 20 mg tail clips, which had been stored in 90% EtOH, either with the DNeasy Tissue kit (Qiagen; Valencia, CA), following manufacturer's instructions for rodent tails or using a standard phenol/chloroform extraction protocol (Sambrook et al., 1989). To amplify the cytochrome *b* gene (cyt *b*), we used the following primers from Carstens et al. (2005): tRNA-Threonine (5′-TTCAGCTTACAAGGCTGATGTTTT-3′); tRNA-Glucine (5′-TTGTATTCAACTATAAAAA C-3′); forward internal 5′-TCCACCCATACTTTTCT TATAAAGA-3′; reverse internal 5′-TAATTAGTGGA TTTGCTGGTGTAA-3′. Amplicons were purified using polyethylene glycol precipitation, and sequencing reactions were performed with the BigDye Kit version 2.0 (Applied Biosystems; Foster City, CA) with 20–40 ng of PCR product in 15 μl volumes. CentriSep columns (Princeton Separations; Adelphia, NJ) were used to filter sequencing reactions, and samples were run on an ABI 377 automated sequencer using 5% Long Ranger polyacrylamide gels. Cyt *b* was sequenced in both the 5′ and 3′ directions, and edited and aligned with Sequencer 3.0 (GeneCodes; Ann Arbor, MI). Sequences were deposited in GenBank (Appendix).

2.2. Phylogenetic analyses

We generated maximum parsimony (MP) and maximum likelihood (ML) estimates of the phylogeny to identify major clades and to test phylogenetic hypotheses in *Dicamptodon*. We pruned all redundant haplotypes, and performed searches with PAUP* 4.0 (Swofford, 2002), both with *A. mexicanum* as an outgroup and without an outgroup. The MP searches were

conducted with stepwise-addition starting trees (150 random-addition replicates) and TBR branch-swapping. For the ML analysis, we used Dt-Modsel (Minin et al., 2003) to select a model of sequence evolution; this method incorporates fit, a penalty for over-parameterization, and performance into model selection. It also selects simpler models than other automated model-selection methods (e.g., Modeltest; Posada and Crandall, 1998) and estimates phylogeny as accurately as more complex models (Abdo et al., 2005). We then conducted heuristic searches under ML with the chosen model, and TBR branch-swapping, and 10 random-addition sequence replicates. Nodal support for both the MP and ML tree was assessed using non-parametric bootstrap values (Felsenstein, 1985), computed from 200 replicates. We estimated the phylogeny of *Dicamptodon* with *A. mexicanum* as an outgroup using both ML and MP. For the MP analysis, we translated nucleotide sequence into amino acids, and conducted a MP search on these data to attempt rooting based only on slowly evolving characters.

2.3. Bayesian hypothesis testing

Recent advances in phylogenetic methods allow evolutionary biologists to conduct tests of a priori hypotheses with several approaches. However, regardless of the method used, testing hypotheses shown in Fig. 2 requires rooting the *Dicamptodon* phylogeny. Although the family Ambystomatidae is likely to be the sister taxon to Dicamptodontidae (Larson, 1991; Larson and Dimmick, 1993), fossil evidence (Estes, 1981) suggests that *Dicamptodon* have been independent of the *Ambystoma* lineage for a considerable period of time and may be too divergent to serve as a reliable root. We explored two approaches for rooting our phylogeny estimate: outgroup rooting and rooting under the molecular clock hypothesis. We examined the effectiveness of rooting the phylogeny with *A. mexicanum* by conducting Bayesian estimation (using MrBayes; Huelsenbeck and Ronquist, 2001) and determining the posterior probability of each of 15 possible root placements. In addition, we conducted a likelihood-ratio test (Felsenstein, 1988) of the molecular clock hypothesis and conducted Bayesian searches under a strict molecular clock to determine the posterior probability of each of the 15 possible root placements for a four-taxon tree. In each analysis, we assumed each of the four species to be monophyletic groups, based on the results of our ML tree, and filtered the posterior distribution of topologies from Bayesian searches described below with filters that corresponded to each possible root.

In addition, we used MrBayes (Huelsenbeck and Ronquist, 2001) to assess the posterior probability of each of the four a priori hypotheses described in the introduction: ancient vicariance, inland dispersal-north, and inland dispersal-south hypotheses for *D. aterrimus*, and Pleistocene-speciation hypothesis for *D. copei*. Achieving stationarity with respect to topology is critical for Bayesian hypothesis testing because we are assessing topological predictions. The topology parameter may be particularly susceptible to non-stationarity (Huelsenbeck et al., 2002), so we employed a stationarity test used by Carstens et al. (2004), which is similar to one proposed by Huelsenbeck et al. (2002). We conducted four independent heated runs (each composed of four Metropolis-coupled chains) and started each run with a different random tree. We ran the chains for 3.1×10^6 generations and sampled trees every 1000 generations. If the four independent runs have each converged on the true joint posterior-probability distribution, the four samples of trees should represent independent samples drawn from that distribution. To assess this expectation statistically, we saved the last 3000 trees from each run and computed the symmetric-difference distance between each tree in the sample and our ML tree using PAUP* 4.0. We then conducted a standard ANOVA on the four groups of tree-to-tree distances to assess whether the four chains could have been drawn independently from the same underlying joint posterior-probability distribution. While a non-significant result for this test would not guarantee that the runs have reached stationarity with respect to topology, it would provide much stronger evidence of such than would the standard examination of $\ln L$ plots (Huelsenbeck et al., 2002). To complete the hypothesis test, we then imported the sample of trees from the Bayesian analysis into PAUP* and filtered them with constraint trees predicted by each of the a priori hypotheses. The proportion of trees in the sample consistent with the topology predicted by each hypothesis is the Bayesian conditional probability that the hypothesis is correct.

3. Results

3.1. Sequencing

We sequenced all of cyt *b* and a portion of the tRNA(Thr), corresponding to positions 14,109–15,249 of the *A. mexicanum* mitochondrial genome, for 68 individual giant salamanders. We translated the nucleotide data to amino acids, checked for stop codons, and aligned the amino acids with other salamander cyt *b* sequences to verify that the pattern of molecular evolution was consistent with the mitochondrial DNA of salamanders and inconsistent with the presence of nuclear pseudogenes. Data from cyt *b* and the tRNA(Thr) were combined into a single data set with a total of 1174 bases. Several individuals had identical haplotypes (Appendix). In the final data set there were six *D. aterrimus*, seven *D. copei*, five *D. ensatus*, and 25

Table 1
Genetic distances

	D. aterrimus	*D. copei*	*D. ensatus*	*D. tenebrosus*	*A. mexicanum*
D. aterrimus	—	0.0999	0.0666	0.0960	1.3726
D. copei	0.0656	—	0.0658	0.0820	1.6416
D. ensatus	0.0503	0.0434	—	0.0589	1.2578
D. tenebrosus	0.0670	0.0572	0.0455	—	1.5946
A. mexicanum	0.2155	0.2223	0.2061	0.2207	—

Shown above the diagonal are genetic distances corrected under the HKY + I + Γ model of sequence evolution in units of substitutions per site. Uncorrected percent sequence divergences are shown below the diagonal.

D. tenebrosus haplotypes. Genetic distances corrected with the HKY + I + Γ model of sequence evolution (see below) as well as uncorrected percent sequence difference are shown in Table 1. Uncorrected divergence ranged from 0.043 to 0.067 within *Dicamptodon*, and from 0.206 to 0.222 between *Dicamptodon* and *Ambystoma*.

3.2. Phylogenetic analyses

We selected the HKY + I + Γ model of sequence evolution using Dt-ModSel (Minin et al., 2003) with equilibrium base frequencies of $\pi_A = 0.311$; $\pi_C = 0.194$; $\pi_G = 0.123$; $\pi_T = 0.371$; transition–transversion ratio = 3.698; proportion of invariable sites = 0.754; and Γ-distribution shape parameter ($\alpha = 1.57$). The ML phylogeny estimate has a likelihood score of $-\ln L = 3189.8281$. When we enforced the molecular clock and conducted a ML search, the resulting tree had a $-\ln L = -3237.8343$. The likelihood-ratio test indicated that we could reject the molecular clock hypothesis ($\delta = 96.0124$; $p < 0.001$). Other than a few relationships within northern *D. tenebrosus*, the MP phylogeny (not shown) is identical to the ML phylogeny (Fig. 3). There is strong bootstrap support for monophyly of haplotypes sampled from each of the four described species [*D. aterrimus* (ML = 100% of the replicates, MP = 100%); *D. copei* (ML = 90%, MP = 99%); *D. ensatus* (ML = 83%, MP = 84%); and *D. tenebrosus* (ML = 94%, MP = 97%)], but little support for relationships among the four species.

3.3. Bayesian hypothesis testing

Genetic distance between *A. mexicanum* and *Dicamptodon* (1.37–1.64 substitutions/site and 21.6–22.2% uncorrected divergence) led us to question the appropriateness of *Ambystoma* as an outgroup (Table 1). We explored this by adding other salamander cyt *b* sequences to the data matrix and estimating the phylogeny with neighbor joining and uncorrected distances as a fast way to explore the sister-group relationship between *Ambystoma* and *Dicamptodon*. In every case, *A. mexicanum* was the sister taxon to *Dicamptodon*, but separated by an extremely long branch (data not shown). Thus, while *A. mexicanum* was the best available outgroup, it may not be a particularly good outgroup. Consequently, we compared the results of three different rooting methods. A ML search of the data, using *A. mexicanum* sequence to root the phylogeny, recovers a paraphyletic *D. ensatus* as the sister taxon to a group containing all other Dicamptodontidae (Fig. 4A). The phylogram illustrates the discrepancy between the length of branches within the ingroup compared to the length of the outgroup branch. A strict consensus of the most parsimonious trees in the parsimony search of amino acids, again using *A. mexicanum* sequence to root the phylogeny, placed *D. copei* outside a clade comprising all other dicamptodontids (Fig. 4B). We used Bayesian methods, again with *A. mexicanum* as an outgroup, to estimate the posterior probabilities for each possible rooting of the genus, and found little support for any of the root placements (*D. tenebrosus* = 0.464; *D. ensatus* = 0.391; *D. copei* = 0.072; and *D. aterrimus* = 0.027). There is therefore little support for any root using the outgroup strategy.

Huelsenbeck et al. (2002) demonstrated that a Bayesian rooting under a clock provides reliable roots, and that this conclusion is robust to violations of the clock assumption. Therefore, we used MrBayes to compute the probability of each of the 15 possible root placements assuming monophyly of each species. These analyses indicated that *D. aterrimus* represents the earliest divergence in the genus and is the sister lineage to the other giant salamanders (Fig. 5). This placement of *D. aterrimus* is consistent with previous work using allozymes (which also found lack of an appropriate outgroup; Good, 1989), and is also the only strong signal for any rooting. We thus consider *D. aterrimus* to be the sister taxon to the rest of the genus for hypothesis testing, but stress that this placement is tentative.

The four independent Bayesian runs had average tree-to-tree distances from the ML tree of 31.34, 31.56, 32.05, and 31.56. The ANOVA indicated that these values were not significantly different ($F_{OBS} = 1.504$; $0.1 > p > 0.05$), a result which we interpreted as evidence that independent Metropolis-coupled MCMC chains were sampling topologies from the same joint posterior-probability distribution and have likely achieved stationarity with respect to topology. We discarded trees from the first 100,000 burn-in generations, and com-

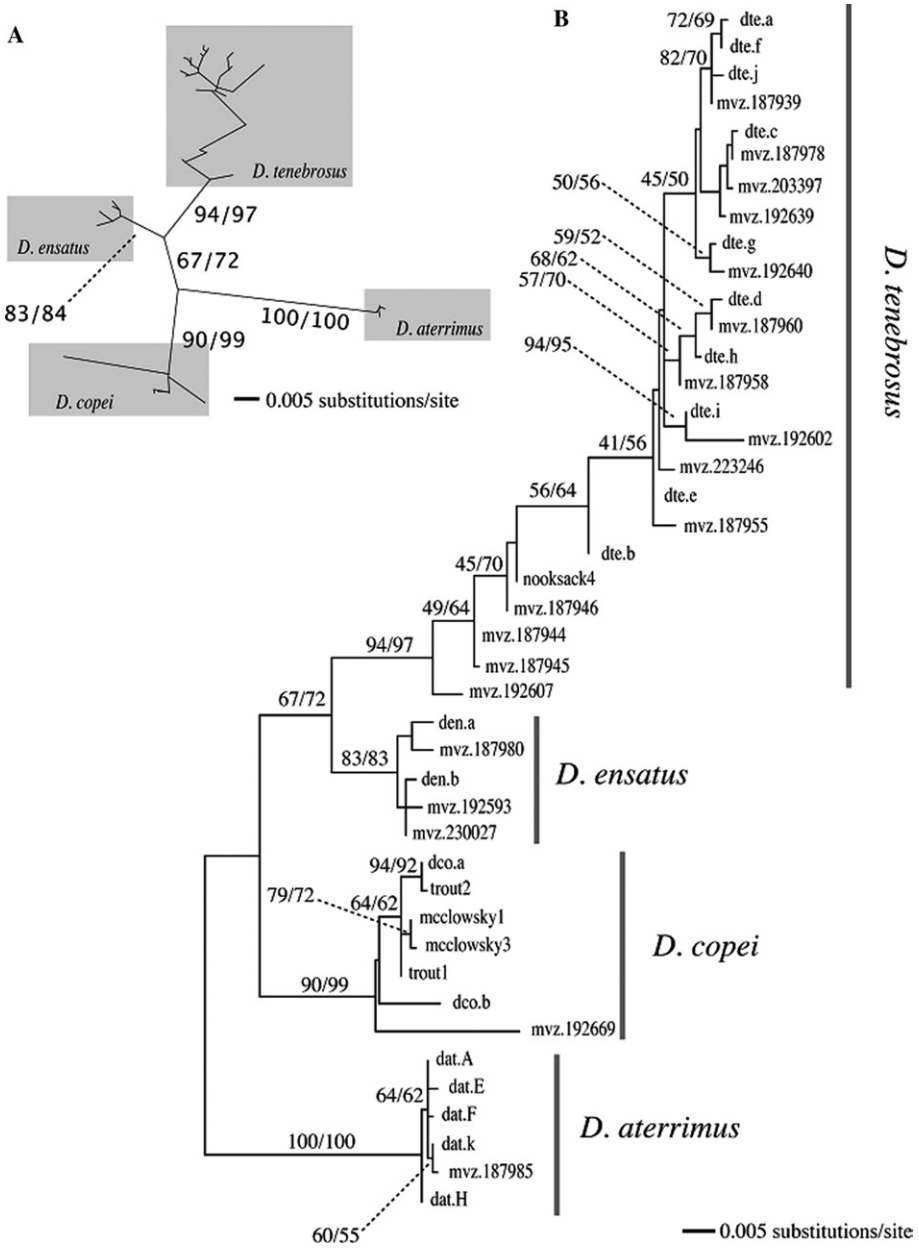

Fig. 3. The optimal ML phylogeny for the salamander genus *Dicamptodon*. An unrooted phylogeny (A) demonstrates the long branches between the four species. A ML phylogeny, rooted with *D. aterrimus* (B) from 43 unique mtDNA haplotypes of 1174 bp of cyt *b* and tRNA-threonine with $\ln L = 3189.8281$. Numbers on branches are ML (above) and MP (below) bootstrap support of nodes retained in >50% of 200 replicates.

bined 3000 trees from each run into a set of 12,000 trees that were used to test the a priori hypotheses. For *D. aterrimus*, we could reject the inland dispersal north hypothesis ($p < 0.0001$) and the inland dispersal south hypothesis ($p < 0.0001$), but not the ancient vicariance hypothesis ($p = 0.9936$). For *D. copei*, we found that we could reject the hypothesis of Nussbaum (1976) that proposed Pleistocene isolation from northern *D. tenebrosus* ($p < 0.0001$) but could not reject the monophyly of either *D. copei* ($p = 1.0$) or *D. tenebrosus* ($p = 1.0$). These results suggest that speciation within *Dicamptodon* was largely shaped by pre-Pleistocene events.

4. Discussion

Our data provide additional insight into the systematics of the salamander genus *Dicamptodon* and the biogeography of the PNW region by rejecting speciation hypotheses that the genetic structure of the genus was primarily shaped by post-Pleistocene events. For both *D. aterrimus* and *D. copei*, we were unable to reject hypotheses that posited pre-Pleistocene isolation. The finding of monophyly in *D. copei*, which at one time was considered polyphyletic (Daugherty et al., 1983), as well as the other species, suggests that these lineages

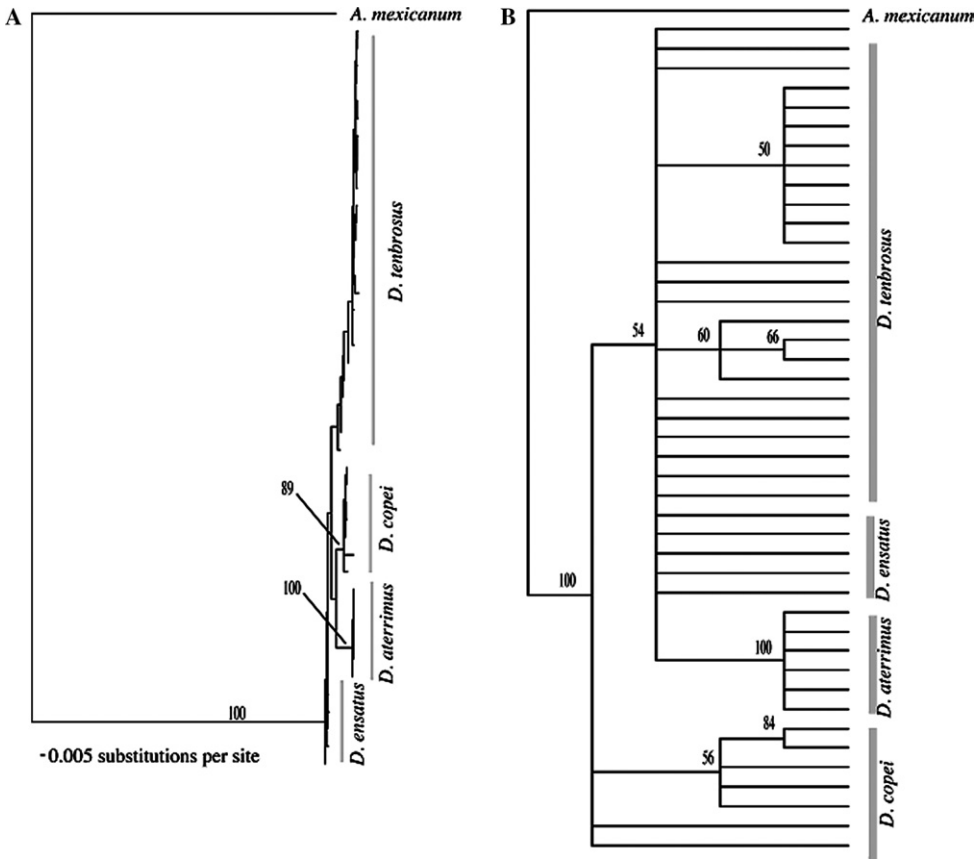

Fig. 4. Estimates of the *Dicamptodon* phylogeny using *A. mexicanum* as the outgroup. A maximum likelihood search of the sequence data showing a paraphyletic *D. ensatus* as the sister taxon to a group comprising all other dicamptodontids (A), a strict consensus of the most parsimonious trees from a search of amino acid data showing a paraphyletic *D. copei* outside a clade containing all other Dicamptodontidae (B).

have been on separate evolutionary trajectories for a significant amount of time. The rejection of the inland dispersal hypotheses for *D. aterrimus* and the strong support for the ancient vicariance hypothesis is congruent with the pattern that has been observed in other mesic-forest amphibians (including the tailed-frog, *Ascaphus trueil A. montanus* [Neilson et al., 2001] and the *Plethodon vandykeil P. idaohensis* complex [Carstens et al., 2004]).

Our findings, combined with previous research on amphibian members of this mesic-forest ecosystem, strongly suggest that *Dicamptodon* was once widespread throughout the PNW during the Miocene. Physical evidence such as dicamptodontine fossils and trackways occurring as far east as Montana and North Dakota further support this conclusion (Estes, 1981; Peabody, 1954, 1959). While pre-Pleistocene xerification in the Columbian Plateau was apparently responsible for separating inland and coastal populations, there was a recent opportunity for gene flow between the two regions during the Pleistocene along a northern corridor of mesic forests during the glacial maxima approximately 25,000–10,000 years ago (Barnosky et al., 1987; Richmond et al., 1965). Such a corridor has been invoked to explain subtle morphological similarities between inland and north-coastal populations of *Dicamptodon* (Nussbaum, 1976). However, the mesic-forest amphibians of the PNW are probably limited in their ability to disperse long distances overland because they are either stream-breeding (*Dicamptodon*, *Ascaphus*) or closely associated with seeps and streams (*P. vandykeil P. idahoensis*). While phylogenetic patterns suggest inland dispersal along a northern corridor in several plant species and small mammals (reviewed in Brunsfeld et al., 2001), there is no genetic evidence for such a pattern in *Dicamptodon*, *Ascaphus*, or the *Plethodon vandykeil P. idahoensis* complex.

Rejection of the Pleistocene-speciation hypothesis for *D. copei* suggests that northern *D. tenebrosus* and *D. copei* are not as closely related as once thought, and that speciation of *D. copei* occurred earlier than previously hypothesized (Nussbaum, 1976). Genetic distance between *D. copei* and other *Dicamptodon* supports earlier speculation by Daugherty et al. (1983) that *D. copei* is an ancient lineage, and offers clues about the relative timing of divergence events. High divergences between *D. copei* and other members of the genus suggest that *D. copei* diverged at approximately the same

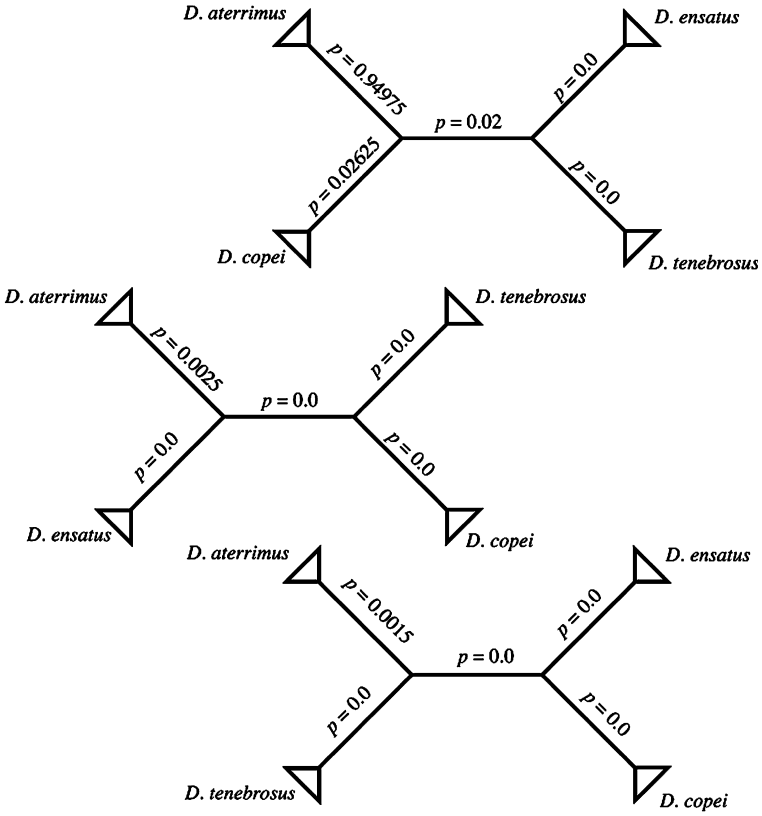

Fig. 5. Bayesian posterior probabilities of each of the 15 possible root placements on a four-taxon tree. The root placement with the highest posterior probability is the branch leading to *D. aterrimus*.

time as *D. aterrimus* was isolated from coastal *Dicamptodon*. The basic premise of Nussbaum's hypothesis may be correct but would require that populations in the northern Cascades, from which the ancestors of *D. copei* diverged, were unable to escape advancing glaciers and that modern populations of *D. tenebrosus* have recently expanded into the north Cascades. Testing this hypothesis will require additional *D. tenebrosus* sampling, explicit phylogeographic modeling, and coalescent-based hypothesis testing following Knowles (2001) and Carstens et al. (2005).

Support for the sister-group relationship between *D. tenebrosus* and *D. ensatus* is high in our analyses, as indicated by MP (72) and ML (67) bootstrapping and Bayesian posterior probability ($p = 0.9483$). No obvious geographic barrier exists between *D. tenebrosus* and *D. ensatus* that would suggest allopatric speciation. However, the split occurs along the 'North Coast Divide' (Nussbaum, 1976), a low ridge that delineates the southern range limit in some taxa and divides a variety of species into subspecies (reviewed in Good, 1989). Such taxa include the transition of mountain kingsnake subspecies *Lampropeltis zonata zonata* to the intergrade zone of *L. z. zonata* x *multicincta* (McGurty, 1988; Zweifel, 1952) and the boundary between the Northern Alligator lizard subspecies *Elgaria coerulea coerulea* and *E. c. shastensis* (Smith, 1995). Recent geologic activity in this region of northern California is characterized by erosion (Wahrhaftig and Birman, 1965), and it may be that the North Coast Divide delimited the boundary of a coastal refuge for *D. ensatus* during the Pleistocene. *D. ensatus* has close associations with the same redwood forest (*Sequoia sempervirens*) habitat as fossil dicamptodontine salamanders and is thought to be more similar morphologically to ancestral *Dicamptodon* than are other extant species (Nussbaum, 1976). It may be that *D. ensatus* persisted throughout the Pleistocene in a southern refugium containing redwood habitat similar to that occupied by ancestral *Dicamptodon*, while *D. tenebrosus* was isolated in separate refugia to the north. Thus, the secondary contact between these forms in northern California (Good, 1989) is the result of recent range expansion from their respective refugia. Again, testing these hypotheses will require additional sampling and explicit coalescent modeling.

Understanding the geological events that contributed to the speciation of extant lineages is one of the primary goals of biogeographic research. This research is complicated when the taxa in question are not closely related to other extant species, but advances in computational phylogenetics allow for the testing of hypotheses even when an appropriate outgroup is not available. As a result, our estimate of the *Dicamptodon* phylogeny permits the following hypothesized history. During the Pliocene (5–2 mya), the ancestors of *D. aterrimus* were isolated from

other *Dicamptodon* by xerification of the Columbia basin following the orogeny of the Cascades. By the end of the Pliocene or early Pleistocene, the ancestors of *D. copei* were isolated from other coastal *Dicamptodon* (~2 mya), and evolved obligate neoteny in a pluvial environment, likely as described by Nussbaum (1976). The remaining coastal *Dicamptodon* lineages were divided into at least two populations by Pleistocene climatic change; the southern populations evolved into *D. ensatus* and northern populations evolved into *D. tenebrosus*. The support of the ancient vicariance hypothesis for *D. aterrimus* and other mesic-forest amphibians provides evidence that amphibian species in the PNW were similarly affected by pre-Pleistocene events.

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Appendix

Locality information, GenBank accession numbers, and museum voucher numbers (if applicable) for samples used in this study

Species	Pop. number	Number sequenced	Unique haplotype labels	County and state; locality information and museum voucher numbers; GenBank accession numbers
D. tenebrosus	1	5	dte.b, nooksack4	Whatcom Co, WA; trib at Nooksack Fls; AY734585
	2	1	dte.b	King Co, WA; Lk Forest Park, MVZ 187972; AY734571
	3	3	dte.b	Multonomah Co, OR; Oneonta Gorge, MVZ 187949–51; AY734572, AY734614, and AY734615
	4	3	dte.h	Tillamook Co, OR; Kilchis River Park, MVZ 192583, 192589–90; AY734582
	5	3	dte.187944, dte.187945, dte.187946	*Wasco* Co, OR; Oak Spgs, MVZ 187944–46; AY734586, AY734598, and AY734587
	6	3	dte.d, dte.187960	Benton Co, OR; Fall Crk, MVZ 187959–61; AY734576, AY734590
	7	3	dte.e, dte.223246	Lane Co, OR; Lookout Crk, MVZ 223245–46, 223248; AY734578, AY734594, and AY734618
	8	2	dte.e, dte.187955	*Lane* Co, OR; N Fork Willamette Riv, MVZ 187954–55; AY734577, AY734588
	9	1	dte.187958	Douglas Co, OR; Smith Riv Fls, MVZ 187958; AY734589
	10	3	dte.g, dte.192607	Josephine Co, OR; Thompson Crk, MVZ 192606–08; AY734581, AY734592, and AY734619
	11	2	dte.j	Jackson Co, OR; Shoat Spgs; AY734584
	12	3	dte.i, dte.192602	Del Norte Co, CA; Rowdy Crk, MVZ 192601–03; AY734583, AY734591, and AY734620
	13	4	dte.a, dte.187939, dte. f	Siskiyou Co, CA; Wingate Crk, MVZ 187933-34; AY734568, AY734613; O'Neill Crk, MVZ 187939–40; AY734597, AY734579
	14	3	dte.c	Trinity Co, CA; Price Crk, MVZ 187929–31; AY734573, AY734616, and AY734617
	15	2	dte.c, dte.f	Shasta Co, CA; approx 2 mi E of Delta, MVZ 192613; AY734574; Lk Shasta; Lower Brock Crk, MVZ 161132; AY734580
	16	5	dte.c, dte.187978, dte.192639, dte.192640, dte.203397	Mendocimo Co, CA; Drive-Thru-Tree at Leggett, MVZ 187978; AY734596; Hwy 1 at Crk between Fort Bragg and Rockport, MVZ 192579; AY734575; Hwy 1at 1.4 mi S of Little Riv, MVZ 192639–40; AY734595, AY734593; Signal Port Crk, MVZ 203397; AY734599
D. ensatus	17	2	den.a	Mendocino Co, CA; Hwy 1 at Quinliven Gulch, MVZ 202532; AY734600; Hwy 1 at Getchell Gulch, MVZ 202831; AY734621

Appendix (*continued*)

Species	Pop. number	Number sequenced	Unique haplotype labels	County and state; locality information and museum voucher numbers; GenBank accession numbers
	18	2	den.a	Sonoma Co, CA; Skaggs Spgs, MVZ 187979; AY734601; Redwood Canyon, MVZ 238149; AY734622
	19	1	den.187980	Marin Co, CA, MVZ 187980; AY734603
	20	1	den. 230027	San Mateo Co, CA; Jct Purisima Crk Rd and Higgins Rd, MVZ 230027; AY734605
	21	4	den.b, den.192593	Santa Cruz Co, CA; Boulder Crk, MVZ 192593–96; AY734604, AY734602, AY734623, and AY734624
D. copei	22	2	trout1, trout2	Skamania Co, WA; Trout Crk; AY734610, AY734611
	23	2	mcclowsky1, mcclowsky 3	Skamania Co, WA; McClowskey Crk; AY734608, AY734609
	24	3	dco.b	Grays Harbor Co WA; Merriman Crk, MVZ 197776–77, 207659; AY734607, AY734627, and AY734628
	25	2	dte.b, dco.192669	Mason Co, WA; 0.3 mi S Mohrweis, Headwaters Weaver Crk, MVZ 192669; AY734612; Save Crk, MVZ 223515; AY734570
	26	3	dco.a	Multnomah Co, OR; Wahkeena Fls, MVZ 192670–72; AY734606, AY734625, and AY734626
D. aterrimus	27	4	dat.A, dat k	Latah Co, ID; Eldorado Gulch; AY728902, AY728912
	28	3	dat.H	Shoshone Co, ID; AY728909
	29	3	dat.F	Clearwater Co, ID; AY728907
	30	3	dat.E	Idaho Co, ID; AY728906
	31	5	mvz.187985	Valley Co, ID; Roaring Crk, MVZ 187984–85, AY728952

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